Issued October 11, 1912.

United States Department of Agriculture,

BUREAU OF CHEMISTRY—Circular No. 103.

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STUDIES ON CHICKEN FAT.[1]

III. INFLUENCE OF TEMPERATURE ON THE LIPOLYSIS OF ESTERS. IV. THE HYDROLYSIS OF CHICKEN FAT BY MEANS OF LIPASE.

By M. E. Pennington and J. S. Hepburn, *Food Research Laboratory.*

V. THE OCCURRENCE OF CATALASE, OXIDASES, AND REDUCTASES IN THE FAT OF THE COMMON FOWL (GALLUS DOMESTICUS).

By Joseph S. Hepburn, *Food Research Laboratory.*

III. INFLUENCE OF TEMPERATURE ON THE LIPOLYSIS OF ESTERS.

Since the acidity of the crude fat increases and the lipase retains its activity in chickens which have been kept at various temperatures for varying periods of time [2] after being killed, it seemed desirable to study the influence of temperatures on the lipolysis of esters. The analytic data of this study are given in Table I. Several fat, old hens were killed, the animal heat was removed by subjecting to a temperature of 0° C. for 24 hours, and they were then kept in a refrigerator for 7 days to permit the lipase to become active; an aqueous lipase solution was prepared according to the technique given in a previous paper.[2]

The determinations proper and the blanks were prepared in exactly the same manner as in the preceding experiments. When the temperature of incubation was that of the chill room or of the freezer, however, flasks of 250 cc capacity were used so that the reaction mixture might congeal without breaking the container. Toluol was used as a bactericide. The flasks were incubated in an incubator at 40° C.; in a refrigerator of 60° to 69° F. (15.6° to 20.6° C.), average 63° F. (17.2° C.); in a mechanically refrigerated chill room at 32° F. (0° C.); and in a mechanically refrigerated freezer at 15° to 20° F.

[1] See U. S. Dept. Agr., Bureau of Chemistry Cir. 75 for I and II.

[2] Pennington and Hepburn. U. S. Dept. Agr., Bureau of Chemistry Cir. 75, pp. 1-7; J. Amer. Chem. Soc. 1912, 34: 210.

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(−9.4° to −6.7° C.). The period of incubation varied between the extremes of 3 days in the incubator and 151 days in the freezer. The contents of the flasks stored in the chill room congealed to some extent, while the contents of those kept in the freezer froze solid. At the end of the period of incubation at low temperatures it was necessary to thaw the contents of these flasks, in order to titrate. Thawing in running water was found to be preferable to thawing in the refrigerator overnight, a period of 16 hours. For example, when incubation in the chill room for 44 days was followed by thawing in the refrigerator, the increase in acidity due to lipase was slightly greater with each of the four esters than when incubation in the chill room for 44 days was followed by thawing in running water; during the short period in the refrigerator sufficient lipolysis occurred to introduce a slight error in the result.

The greatest splitting of the esters by the lipase took place in the incubator, the least in the freezer, and between these two extremes lay the splitting in the refrigerator and in the chill room.

The increase in acidity due to the action of lipase for 3 days in the incubator at 40° C. has been chosen as a standard for comparison. With this are compared the increases in acidity due to the action of the enzym for 3 days in the refrigerator, for 18 days in the chill room, and for 45 days in the freezer. The increase in acidity produced by the lipase during further incubation is influenced by the products formed during the earlier stages of lipolysis, and, therefore, it is better to use in the comparison the results obtained during the shortest period of incubation at each temperature. When the comparison is made of the increases in acidity produced by lipase at various temperatures, and the ratios are expressed throughout on a basis of the action of the enzym for a period of 3 days, the following data are obtained:

The lipolysis of ethyl acetate in the incubator is twice as rapid as in the refrigerator, 15 times as rapid as in the chill room, and 37½ times as rapid as in the freezer. Ethyl butyrate is hydrolyzed by lipase in the incubator 2½ times as fast as in the refrigerator, 12 times as fast as in the chill room, and 40 times as fast as in the freezer. Ethyl benzoate is split by the enzym in the incubator 8½ times as rapidly as in the refrigerator, 25½ times as rapidly as in the chill room, and 255 times as rapidly as in the freezer. The hydrolysis of amyl salicylate by lipase in the incubator is 6½ times as rapid as in the refrigerator, 13 times as rapid as in the chill room, and 97½ times as rapid as in the freezer.

Although the rate of lipolysis is decreased by a lowering of the temperature, yet lipolysis takes place even at the temperature of the freezer when the reaction mixture is frozen solid.

TABLE I.—*Influence of temperature on the lipolysis of esters (Experiment 377).*

Temperature of incubation.	Period of incubation.	Ester.	Acidity expressed as cubic centimeters of tenth-normal sodium hydroxid.		
			Determination proper after incubation.	Blank after incubation.	Increase due to lipase.
	Days.				
Incubator 40° C.	3	Ethyl acetate	1.50	0.25	1.25
		Ethyl butyrate	2.25	.30	1.95
		Ethyl benzoate	1.35	.50	.85
		Amyl salicylate	.85	.20	.65
Refrigerator; maximum temperature, 19.4° C.; minimum temperature, 15.6° C.; average temperature, 17.2° C.	3	Ethyl acetate	.80	.20	.60
		Ethyl butyrate	.95	.15	.80
		Ethyl benzoate	.40	.30	.10
		Amyl salicylate	.30	.20	.10
Refrigerator; maximum temperature, 20.6° C.; minimum temperature, 15.6° C.; average temperature, 17.2° C.	9	Ethyl acetate	1.15	.20	.95
		Ethyl butyrate	1.25	.10	1.15
		Ethyl benzoate	.55	.40	.15
		Amyl salicylate	.35	.25	.10
Chill room, 0° C.; thawed overnight in refrigerator.	18	Ethyl acetate	.65	.15	.50
		Ethyl butyrate	1.00	.10	.90
		Ethyl benzoate	.50	.30	.20
		Amyl salicylate	.50	.20	.30
Chill room, 0° C.; thawed in running water.	44	Ethyl acetate	1.05	.20	.85
		Ethyl butyrate	1.05	.25	.80
		Ethyl benzoate	.60	.40	.20
		Amyl salicylate	.25	.20	.05
Chill room, 0° C.; thawed overnight in refrigerator.	44	Ethyl acetate	1.10	.20	.90
		Ethyl butyrate	1.10	.15	.95
		Ethyl benzoate	.65	.35	.30
		Amyl salicylate	.30	.20	.10
Freezer, −9.4 to −6.7° C.; thawed in running water.	45	Ethyl acetate	.60	.10	.50
		Ethyl butyrate	.75	.00	.75
		Ethyl benzoate	.05	.00	.05
		Amyl salicylate	.15	.05	.10
Freezer, −9.4 to −6.7° C.; thawed in running water.	98	Ethyl acetate	.55	.05	.50
		Ethyl butyrate	.85	.05	.80
		Ethyl benzoate	.15	.00	.15
		Amyl salicylate	.10	.00	.10
Freezer, −9.4 to −6.7° C.; thawed in running water.	151	Ethyl acetate	.50	.10	.40
		Ethyl butyrate	.80	.10	.70
		Ethyl benzoate	.10	.00	.10
		Amyl salicylate	.25	.10	.15

IV. THE HYDROLYSIS OF CHICKEN FAT BY MEANS OF LIPASE.

Since lipase occurs in the crude fat of chickens and gives rise to the post-mortem hydrolysis of the fat, the following experiments were made to demonstrate the lipolysis of chicken fat in vitro. The crude fat was extracted in a Soxhlet extractor with ethyl ether, and the fat was recovered by distilling off the solvent. The tissues which remained in the thimble of the extractor were placed in a vacuum desiccator to free them from ether, then they were triturated in a mortar with sand, and extracted with glycerol for a period of 3 days, triturating at intervals. During the extraction toluol was used as a bactericide. The extract was forced through a muslin filter. The quantity of glycerol used for extraction was so regulated that the total volume of the filtrate in cubic centimeters was exactly 10 times the weight in grams of the tissues used in making the extract. Samples of the extracted fat, 10 grams in weight, were placed in Erlenmeyer flasks of 200 cc capacity, and 10 cc of the glycerol extract was added to each flask. Blank experiments were prepared in a similar manner and immersed in boiling water for 5 minutes to destroy the lipase. Experiments proper and blanks were incubated for 72 hours at 40° C.; toluol was used as a bactericide. After incubation, 50 cc of neutral alcohol and a few drops of phenolphthalein solution were added to each flask, which was then heated on the electric stove until the alcohol boiled briskly, whereupon the free acids were titrated and the acid value of the fat was calculated. The results are as follows:

Acid value of fat.

Number of sample.	Acid value.		
	Determination proper after incubation.	Blank after incubation.	Increase due to lipase.
384	3.45	3.36	0.09
387	1.47	1.14	.33

In spite of the fact that a glycerol extract of lipase was used lipolysis had taken place, as is shown by the increase in acid value due to the action of lipase. When the conditions were made more favorable for the action of the enzym, lipolysis became more active, as is shown by the following data: Experiment 387 was made as outlined above,

but a second set of flasks was also prepared in which were placed not only 10 grams of fat, 10 cc of the glycerol extract, and some toluol, but also 10 cc of distilled water. Both sets of flasks were incubated at the same temperature for the same period of time. In the set containing water, the acid value of the determination proper after incubation was 1.67; of the blank after incubation, 1.11; hence the increase due to lipase was 0.56. The glycerol extract by itself gave rise to an increase in acid value of only 0.33, whereas the glycerol extract, plus its own volume of water, gave rise to an increase in acid value of 0.56. In other words, in the more favorable reaction mixture the same quantity of lipase produced one and seven-tenths as great an hydrolysis as in the less favorable substratum.

V. THE OCCURRENCE OF CATALASE, OXIDASES, AND REDUCTASES IN THE FAT OF THE COMMON FOWL (GALLUS DOMESTICUS).

CATALASE.

Euler [1] has found that both lipase and catalase occur in the fat-tissues of swine and in certain seeds, such as those of the pumpkin and of the castor oil plant. Pennington and Hepburn [2] have demonstrated the occurrence and permanence of lipase in the crude fat of the common fowl, hence the present study was undertaken to determine the occurrence of both lipase and catalase in the crude abdominal fat of chickens, including birds just killed and those kept at various temperatures for varying periods of time after being killed. The crude fat was extracted with tenfold its weight of water, as described in a previous communication from this laboratory.[2]

Twenty-five cubic centimeters of the extract were placed in an Erlenmeyer flask of 200 cc capacity, through the stopper of which passed a tap funnel containing 50 cc of 0.3 normal solution of hydrogen peroxid and a delivery tube; the end of the latter dipped beneath the water of a pneumatic trough, in which was inverted a eudiometer filled with water. The entire apparatus was placed in an incubator at 37.5° C. and was permitted to assume the temperature of the incubator. The hydrogen peroxid solution was then introduced into the flask and the eudiometer was immediately placed over the end of the exit tube. The period of incubation was two hours. The volume of oxygen evolved was corrected for a temperature of 0° C. and a pressure of 760 mm of mercury; in the calculation the following factors were considered: Temperature of the incubator, barometric pressure, tension of the aqueous vapor, and difference in level of the water within and without the eudiometer. In like manner blank determinations were made upon 25 cc portions of the boiled extract. In the tabulated results the figures in the column "Oxygen evolved by catalase" represent the difference between the volume of oxygen liberated by the determination proper and the volume liberated by the blank, both volumes having been reduced to standard conditions of temperature and pressure. The acid value of the crude fat was determined by the method of Pennington and Hepburn.[3]

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[1] Hofmeister's Beitrage zur chemischen Physiologie und Pathologie, 1906, **8** : 1.

[2] U. S. Dept. of Agr., Bureau of Chemistry Cir. 75, pp. 1–7; J. Amer. Chem. Soc., 1912, **34** : 210.

[3] Pennington and Hepburn, J. Amer. Chem. Soc., 1910, **32** : 568.

The results of these experiments are given in Table II. Experiments 395 and 423 are chickens just killed and retaining the animal heat; 400 a fowl kept in the refrigerator for a week; 388 a "green struck" bird in the incipient stages of putrefaction, and 399 a chicken in an advanced state of putrefaction. In this series as the period and stage of decomposition increase, a progressive increase in acid value of the crude fat occurs. The same progressive increase in acidity occurs as the period of marketing lengthens, as is shown by the data of experiments 2070 A and B, 2035 A and B, and 2036 A and B.

In experiments 2035 A and B and 2067–4 A, the extract of the crude fat produced hydrolysis of ethyl butyrate; in all the other experiments it gave rise to a splitting of ethyl acetate, butyrate, and benzoate, and of amyl salicylate. Hence lipase was present in all the samples examined, a total of 30. Every one of these samples contained catalase also. Therefore, lipase and catalase accompany each other in the fat of the common fowl, whether just killed, in various stages of marketing, or in incipient or advanced state of putrefaction. While the acidity of the crude fat and the activity of the lipase increase during the time the chicken is being kept, as was demonstrated in the paper on the occurrence and permanence of lipase, yet the activity of the catalase is apparently more or less independent of the period of holding the birds.

TABLE II.—*Occurrence of catalase in chicken fat.*

Sample No.	Description of sample.	Acid value of crude abdominal fat.	Oxygen evolved by catalase.
			cc
395	Chicken retaining animal heat	0.32	13.00
423	....do	0.14	11.80
400	Chicken kept in refrigerator 7 days; maximum temperature, 60° F., 15.6° C.; minimum temperature, 52° F., 11.1° C.; average temperature, 55.6° F., 13.1° C	0.99	13.85
388	Chicken, wet-packed [1] "green struck" incipient putrefaction	5.14	24.35
399	Chicken kept in room 65° to 80° F.; 18.3° to 26.7° C. for 7 days; advanced putrefaction	15.46	10.30
2070–1 A	Fresh dry-packed southern market chickens of known history at end of railroad haul	2.10	11.65
2070–2 A	Same as 2070–1 A, at commission merchant's 5 days	3.12	22.95
2070–3 A	Same as 2070–2 A, at retail dealer's 2 days	3.12	11.10
2070–4 A	Same as 2070–2 A, at retail dealer's 7 days	4.84	13.80
2070–1 B	Fresh wet-packed southern market chickens of known history at end of railroad haul	3.29	11.70
2070–2 B	Same as 2070–1 B, at commission merchant's 5 days	4.79	43.80
2070–3 B	Same as 2070–2 B, at retail dealer's 2 days	4.66	12.80
2070–4 B	Same as 2070–2 B, at retail dealer's 7 days	6.96	6.50
2067–4 A	Fresh dry-packed southern market chickens of known history, at commission merchant's 4 days and retail dealer's 7 days	6.09	7.75

[1] "Dry packing" involved the removal of the feathers by dry picking, and the extraction of the animal heat by subjecting the freshly killed bird to dry cold air, at a temperature of 0° C. for 24 hours. Cold-air refrigeration was maintained throughout marketing whether a period of holding hard frozen had or had not intervened.

"Wet packing" involved dry picking the feathers, then immersing the carcass in cold water, water and ice, and finally in cracked ice, in which it was hauled to market, and kept while marketing. When wet-packed birds were stored hard frozen, however, they were first allowed to drain and dry, then were frozen solid and kept in a freezer until they were again placed on the market, when they were kept under air-chilled refrigeration until thawed. After that they were kept in cracked ice, under the usual procedure.

TABLE II.—*Occurrence of catalase in chicken fat*—Continued.

Sample No.	Description of sample.	Acid value of crude abdominal fat.	Oxygen evolved by catalase.
			cc.
2035-5 A	Dry-packed southern market chickens of known history; hard frozen 9 months	1.62	6.05
2035-6 A	Same as 2035-5 A at commission merchant's 2 days	3.47	2.85
2035-7 A	Same as 2035-6 A, at retail dealer's 5 days	3.28	11.05
2035-8 A	Same as 2035-6 A, at retail dealer's 7 days	6.38	19.50
2035-5 B	Wet-packed southern market chickens of known history; hard frozen for 9 months	2.13	6.25
2035-6 B	Same as 2035-5 B, at commission merchant's 2 days	2.49	13.05
2035-7 B	Same as 2035-6 B, at retail dealer's 5 days	4.51	12.15
2035-8 B	Same as 2035-6 B, at retail dealer's 7 days	6.93	12.80
2036-5 A	Dry-packed southern market chickens of known history; hard frozen 9 months	2.67	12.05
2036-6 A	Same as 2036-5 A, at commission merchant's 4 days	3.08	7.85
2036-7 A	Same as 2036-6 A, at retail dealer's 2 days	4.47	28.10
2036-8 A	Same as 2036-6 A, at retail dealer's 7 days	5.87	8.60
2036-5 B	Wet-packed southern market chickens of known history; hard frozen 9 months	4.79	16.65
2036-6 B	Same as 2036-5 B, at commission merchant's 4 days	4.56	49.15
2036-7 B	Same as 2036-6 B, at retail dealer's 2 days	3.15	13.40
2036-8 B	Same as 2036-6 B, at retail dealer's 7 days	5.17	28.35

OXIDIZING ENZYMS.

Since the fat of chickens which are kept hard frozen undergoes an oxidation similar to that produced in vitro by the action of hydrogen peroxid,[1] it seemed desirable to study the occurrence of oxidizing enzyms in the crude fat of hard-frozen poultry and also to extend the research to include the crude fat of chickens kept at various temperatures for varying periods of time. The recent elaborate treatise of Kastle on The Oxidases and Other Oxygen-catalysts Concerned in Biological Oxidations [2] renders it unnecessary to discuss the literature on oxidases.

The crude fat was extracted with ten times its weight of water in the same manner as the aqueous extracts were prepared for the study of lipase and catalase. For the determination of oxidase, 50 cc of the aqueous extract of the crude fat were mixed in a 100-cc flask with 1 cc of a solution of one of the five reagents, pyrogallol, hydroquinone, pyrocatechin, *p*-phenylene diamin, and *p*-phenylene diamin plus α-naphthol. Two per cent solutions of the polyhydric phenols and of *p*-phenylene diamin were used. The indophenol oxidase reagent was prepared by adding 1.08 grams of *p*-phenylene diamin, 1.44 grams of α-naphthol and 3.18 grams of anhydrous sodium carbonate to 100 cc of water; that is, the solution was $\frac{M}{10}$ for the amin and for the naphthol and $\frac{3M}{10}$ for the sodium carbonate. Both the solution of *p*-phenylene diamin and of the indophenol reagent contained a sediment, and only the clear supernatant solution was used in testing for the enzyms.

[1] Hepburn, U. S. Dept. Agr., Bureau of Chemistry Cir. 75, p. 8; J. Amer. Chem. Soc., 1912, 34: 218.
[2] U. S. Treasury Dept., Hygienic Laboratory Bul. 59.

In every case blank experiments were carried out by boiling 50-cc portions of the extract, permitting to cool to the temperature of the room, and adding the reagents as in the determinations proper. For the determination of oxidase plus peroxidase, determinations proper and blanks were prepared as above, but to each flask was added 1 cc of a 3 per cent solution of hydrogen peroxid. The flasks containing the reaction mixtures were incubated at 40° C. for 18 hours. Toluol was used as a bactericide.

The reaction was considered positive in each case when (1) a color developed in the determination proper while the blank remained colorless; (2) the color of the determination proper was distinctly more pronounced than the color of the blank. In the experiments, to which hydrogen peroxid had not been added, oxidase alone caused the development of a color; in the experiments to which hydrogen peroxid had been added, both oxidase and peroxidase could give rise to the color. The two sets of experiments, however, were carried out side by side, and it thus became possible to compare the intensity of the color produced by the extract with any given reagent with and without the addition of hydrogen peroxid; a more intense color in the flask containing peroxid would demonstrate the presence of peroxidase which had produced the excess of color over and above that produced by the oxidase in the absence of hydrogen peroxid.

The samples examined included a chicken retaining the animal heat, one kept in a mechanically refrigerated chill room at 0° C., one kept in the refrigerator, one in an advanced stage of putrefaction, birds kept hard frozen for varying periods of time, and hard-frozen birds at various periods of their marketing. The analytical data are given in Table III. The plus sign (+) signifies a positive reaction between the extract of the crude fat and the reagent, while the minus sign (−) signifies a negative reaction.

Oxidases were present in the 14 samples examined; in 13 of these experiments one or more of the reagents gave a more intense color when hydrogen peroxid was added to the reaction mixture than when it was omitted; therefore, peroxidases were also present, the only experiment in which they could not be detected being Sample 2050–5 A.

It is interesting to note that, of the three polyhydric phenols, pyrogallol and hydroquinone were far more reactive than pyrocatechin, which gave but one response in 10 experiments.

Mention may be made of the fact that in series 2050 a difference in intensity of the color produced with an enzym reagent, by the dry-packed and the wet-packed chickens of the same period of marketing, occurred 7 times, and in 6 of these cases the wet-packed birds produced the more intense color and therefore contained the more active enzym.

TABLE III.—*Occurrence of oxidases and reductases in chicken fat.*

Sample No.	Description.	Oxidizing enzyms.										Reductases.	
		Pyrogallol.		Hydroquinone.		Pyrocatechin.		*p*-phenylene diamin.		*p*-phenylene diamin plus *a* naphthol.		Methylene blue plus 1 per cent sodium fluorid.	Methylene blue plus formaldehyde.
		Without hydrogen peroxid.	With hydrogen peroxid.	Without hydrogen peroxid.	With hydrogen peroxid.	Without hydrogen peroxid.	With hydrogen peroxid.	Without hydrogen peroxid.	With hydrogen peroxid.	Without hydrogen peroxid.	With hydrogen peroxid.		
423	Chicken retaining animal heat	+	+	−	+	−	−	....	....	−	−	−	−
429	Chicken kept in chill room at 32°F., 0°C. for 15 days	+	+	−	−	−	−	....	....	....	....	−	−
426	Chicken kept in refrigerator 8 days; average temp. 53.9° F., 12.2° C.; maximum temp. 59.0° F., 15.0° C.; minimum temp. 48.0° F., 8.9° C.	−	+	+	+	....	....	....	....	....	....	−	−
425	Chicken kept in room 8 days; average temp. 68.2° F., 20.1° C.; maximum temp. 79.0° F., 26.1° C.; minimum temp. 48.0° F., 8.9° C.; advanced putrefaction	+	+	+	+	−	−	....	−	....	....	−	−
421	Chickens hard frozen for 23 months; history previous to freezing unknown	....	....	+	+	....	....	....	....	....	....	+	+
419	Chicken of known history hard frozen for 36 months	....	+	....	+	....	+	....	−	....	−	....	....
427	Chickens hard frozen for 63 months; history previous to freezing unknown	+	+	+	....	....	....	....	....	....	....	−	+
430	Chicken hard frozen 63 months; history previous to freezing unknown	....	....	....	....	....	....	....	....	....	....	−	−
2050–5 A	Dry-packed southern market chickens of known history; hard frozen 9 months	−	....	+	+	....	....	....	....	....	....	....	....
2050–6 A	Same as 2050–5 A, at commission merchant's 4 days	+	+	+	+	−	−	+	+	+	+	....	....
2050–7 A	Same as 2050–6 A, at retail dealer's 3 days	−	+	+	+	....	....	−	−	....	....	−	−
2050–8 A	Same as 2050–6 A, at retail dealer's 7 days	+	....	−	+	....	....	....	....	−	−	....	....
2050–5 B	Wet-packed southern market chickens of known history; hard frozen 9 months	−	+	+	+	....	....	....	+	....	....	....	....
2050–6 B	Same as 2050–5 B, at commission merchant's 4 days	+	+	−	+	....	....	....	....	....	....	....	....
2050–7 B	Same as 2050–6 B, at retail dealer's 3 days	+	+	+	+	−	....	−	−	+	+	−	−
2050–8 B	Same as 2050–6 B, at retail dealer's 7 days	+	....	−	+	....	....	....	....	....	....	....	....
2053–5 A	Dry-packed southern market chickens of known history; hard frozen 9 months	....	....	....	....	....	....	....	....	....	....	−	−
2053–6 A	Same as 2053–5 A, at commission merchant's 4 days	....	....	....	....	....	....	....	....	....	....	−	−
2053–7 A	Same as 2053–6 A, at retail dealer's 3 days	....	....	....	....	....	....	....	....	....	....	−	−
2053–8 A	Same as 2053–6 A, at retail dealer's 7 days	....	....	....	....	....	....	....	....	....	....	−	−
2053–5 B	Wet-packed southern market chickens of known history; hard frozen 9 months	....	....	....	....	....	....	....	....	....	....	−	−
2053–6 B	Same as 2053–5 B, at commission merchant's 4 days	....	....	....	....	....	....	....	....	....	....	−	−
2053–7 B	Same as 2053–6 B, at retail dealer's 3 days	....	....	....	....	....	....	....	....	....	....	−	−
2053–8 B	Same as 2053–6 B, at retail dealer's 7 days	....	....	....	....	....	....	....	....	....	....	+	−

REDUCTASES.

In preliminary tests for reductase by means of methylene blue, the blank experiment was frequently reduced completely while the determination proper was but partially reduced. It therefore became necessary to add a bactericide to the substratum, and the following technique was finally adopted: The crude abdominal fat of fresh chickens and of birds kept at various temperatures for varying periods of time was extracted with tenfold its weight of water in the manner already described.

Nineteen cubic centimeters of this extract were mixed with 0.2 gram of sodium fluorid, and 1 cc of methylene blue solution. The solution was poured into a one-half ounce bottle until it overflowed and the ground glass stopper was inserted in such a manner that no air was contained in the bottle. This reaction served for the detection of simple reductases.

Nineteen cubic centimeters of the extract were mixed with 1 cc of methylene blue-formalin solution and the resulting solution was placed in a bottle in the manner just described. This reaction served for the detection of aldehyde reductases.

Blank experiments were made on the boiled extract with both reagents; all the determinations proper and blanks were incubated at 40° C. for 18 hours.

The methylene blue solution was prepared by mixing 5 cc of saturated alcoholic solution of methylene blue and 195 cc of water. The methylene blue-formalin solution was prepared by mixing 5 cc of saturated alcoholic solution of methylene blue, 5 cc of formalin (40 per cent formaldehyde) and 190 cc of water. These reagents were, therefore, prepared as directed by Schardinger.[1]

The results obtained are set forth in Table III, where the plus sign (+) shows reduction of the determination proper, and the minus sign (−), absence of reduction in the determination proper. In no case was a blank experiment reduced. In 2 of the 17 experiments with methylene blue, plus sodium fluorid, reduction of the determination proper occurred, and likewise 2 of the 17 experiments with methylene blue-formalin showed reduction of the determination proper. Therefore, both species of reductase may occasionally occur in the crude fat.

CONCLUSIONS.

(1) Catalase and lipase always occur in the crude fat of chickens.

(2) The activity of the catalase is apparently more or less independent of the period of keeping of the chicken after death.

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[1] Zts. Nahr. Genussm., 1902, 5: 1113.

(3) Oxidases always occur in the crude fat.

(4) Peroxidases usually and probably always occur in the crude fat.

(5) Reductases may occur in the crude fat.

Approved:

JAMES WILSON,
Secretary of Agriculture.

WASHINGTON, D. C., *June 21, 1912.*

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